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Detection of Oomycide-Insensitive *Phytophthora* Isolates in Maryland Ornamental Nurseries and Mid-Atlantic Landscapes Provide Data for Reconsidering Management Strategies

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Abstract

Five *Phytophthora* species comprising a total of 243 isolates (77 *P. cinnamomi*, 23 *P. citrophthora*, 18 *P. multivora*, 18 *P. pini*, and 107 *P. plurivora*) were screened for sensitivity to mefenoxam, fosetyl-Al, dimethomorph, dimethomorph + ametoctradin, and fluoxastrobin using amended agar assays. Mefenoxam-insensitive isolates were detected within *P. cinnamomi* (4%), *P. multivora* (11%), and *P. plurivora* (12%) even at approximately 2.5× the recommended label rate. These isolates were also insensitive to higher (off-label) concentrations of fluoxastrobin. Concentrations of dimethomorph (400 μg/ml) and dimethomorph + ametoctradin (100 μg/ml) were mostly effective in mycelial growth inhibition, but two *P. plurivora* isolates were insensitive, suggesting that resistance management is required. All mefenoxam-insensitive isolates were sensitive to fosetyl-Al at the label rate. Surprisingly, populations of *P. cinnamomi* from mid-Atlantic oak forests included insensitive isolates. For most species, isolates recovered from asymptomatic hosts (e.g., soil/potting media collected from randomly selected asymptomatic hosts) had a significantly greater relative growth rate when compared with isolates recovered from symptomatic hosts (e.g., isolates recovered from lesions or wilted plants). These findings suggest that mefenoxam and fluoxastrobin should be used sparingly to manage oomycetes in Maryland ornamental nurseries.

Keywords: pesticide resistance, fungicides, mefenoxam, fluoxastrobin

Phytophthora in Nurseries

Phytophthora root rot, crown rot, and foliage blight are common diseases in the ornamental nursery industry, and management of these diseases is challenging (Daughtrey and Benson 2005), particularly because population buildup in a nursery environment can occur quickly due to the presence of a wide range of potential hosts and increased risk of infestation as a result of production practices (e.g., recirculation of irrigation water, reuse of potting media, homogenous potting media, etc.). Generally, nursery management of *Phytophthora* consists of schedule-based fungicide application programs. Although these practices can help protect plants, there is a risk of overuse selecting for resistant populations over time. For this reason, a periodic evaluation of fungicide sensitivity of *Phytophthora* pathogens is necessary.

Fungicides for Oomycete Pathogen Management

A variety of fungicides, also known as oomycides, are used to manage *Phytophthora* pathogens in nurseries. Among them, the acylanilides and alkyl phosphonates are systemic fungicides that have been shown to be superior to other systemic and protectant fungicides in their ability to inhibit disease development after infection, their lack of vulnerability to washing off, and their longer residual activity (Cohen and Coffey 1986). The chemical metalaxyl is an acylanilide fungicide first used in 1977 to control oomycete diseases on a wide variety of crops including ornamentals (Bhat and Browne 2007; Coffey et al. 1984; Cohen and Coffey 1986; Dunn et al. 2010; Hu et al. 2008; Hwang and Benson 2005; Olson and Benson 2011, 2013; Parra and Ristaino 2001; Pérez-Sierra et al. 2011). However, resistance to the fungicide was reported shortly thereafter in the 1980s (Morton and Urech 1987). In 1996, the manufacturer created metalaxyl-M (mefenoxam), a more active isomer of the fungicide, which is similarly applied (Olson and Benson 2013). Unlike metalaxyl and mefenoxam, which are xylem-mobile fungicides, phosphonate fungicides can move in both the xylem and phloem tissues (Cohen and Coffey 1986; Landschoot and Cook 2005; Twizeyimana et al. 2013). The most effective and perhaps well-known phosphonate fungicide is fosetyl-Al, which was also developed in 1977. Although fosetyl-Al is one of the top 10 fungicides used against oomycetes in Maryland over the last decade (USDA NASS 2018), few instances of fungicide resistance have been reported (Landschoot and Cook 2005). Mefenoxam, in spite of its decreased use over that same period of time, was still listed in the top 100 pesticides used in Maryland in 2004 and in the top 150 in 2011.

Study Objectives

A fungicide sensitivity experiment was conducted to explore whether resistance to commonly used fungicides is present and if

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the most frequently used fungicides are still effective against five of the most commonly encountered *Phytophthora* species in Maryland nurseries. We also explored whether there is a relationship between isolate fungicide sensitivity and variables such as isolate genotype, isolate origin (nursery versus forest), isolate source (soil versus infected tissue), host genera, and host symptom (symptomatic versus asymptomatic). The overarching goal of this study was to provide Maryland nursery managers with information about which fungicides are still effective in managing diseases caused by *Phytophthora.*

Isolate Collection

Isolates included in this study are members of five of the most frequently encountered *Phytophthora* species in six Maryland ornamental nurseries in a previous study (Bienapfl and Balci 2014): *P. cinnamomi*, *P. citrophthora*, *P. pini*, *P. plurivora*, and *P. multivora* (*P. citricola* has been split into several species including *P. pini*, *P. plurivora*, and *P. multivora*). Due to their presumed lack of exposure to fungicides, isolates from mid-Atlantic oak forests collected in 2004 and 2012 were included in the study to serve as controls (Balci et al. 2007, 2008; McConnell and Balci 2014). The most frequently encountered species in oak forests were *P. cinnamomi* and *P. plurivora*. A large subset of these isolates (680 from various hosts and substrates) was previously genotyped using amplified fragment length polymorphism (AFLP), and molecular clusters were generated based on their genetic distances (Beaulieu et al. 2017). In the present study, a subset of 243 isolates including forest and nursery isolates (77 *P. cinnamomi*, 23 *P. citrophthora*, 18 *P. pini*, 107 *P. plurivora*, and 18 *P. multivora*) was selected to represent the AFLP clusters of each of the five species.

Fungicide-Amended Agar Assays

All isolates were maintained on 6-cm-diameter Petri dishes with 8 ml of 10% buffered clarified V8 juice agar (10 g of $CaCO_3$ per 1 liter of V8 juice spun down at 4,000 rpm for 10 min, 100 ml of clarified V8 juice in 900 ml of dH_2O with 10 g of agar). For fungicide sensitivity assays, once autoclaved medium had cooled to 50°C, fungicide was added. After fungicide was thoroughly mixed into the medium with a magnetic stirrer, a peristaltic pump was used to pour 8 ml of medium into 6-cm Petri dishes. Petri dishes with unamended agar served as the control. A 5-mm-diameter sterile cork borer was used to transfer one plug from the growing edge of a 5- to 10-day-old isolate onto the center (mycelia facing down) of each Petri dish. Plates then were incubated in the dark at 25°C, and at 72 h the diameter of the mycelial growth (mm) was measured along two perpendicular lines, subtracting the diameter of the plug. Relative growth (RG) was calculated for each isolate by comparing the average diameter measurements to those of controls. Each isolate was tested using three Petri dishes with fungicide-amended agar and three nonamended agar controls. The experiments were repeated twice for all isolates and up to four times if statistically significant differences were detected between the two trials (see below).

Fungicides

Chemicals commonly used to manage oomycetes and several newer products were utilized in the sensitivity assays. Specifically, mefenoxam (Subdue Maxx, 22% active ingredient [a.i.], Syngenta Crop Protection, Greensboro, NC), fosetyl-Al (Aliette WDG, 80% a.i., Bayer Crop Science, Research Triangle Park, NC), dimethomorph (Stature SC, 43.5% a.i., BASF), dimethomorph + ametoctradin (Orvego, 47.1% a.i., BASF), and fluoxastrobin (Disarm 480 SC, 40.3% a.i., Arysta LifeScience North America, Mainland, PA) were used (Table 1). Initially, only one concentration (100 µg/ml a.i.) of mefenoxam and fosetyl-Al was tested to screen all isolates for fungicide sensitivity. These concentrations were selected based on previous studies, although they were 12.5 to 25× higher than the label rate for mefenoxam and 30× lower than that for fosetyl-Al (Chan and Kwee 1986; Farih et al. 1981; Guest 1984; Matheron and Porchas 2000). Isolates were considered sensitive if RG was 0%, intermediately sensitive if RG was >0 to <50%, and insensitive if RG was ≥50% of the control plates (Hwang and Benson 2005). Isolates labeled "insensitive" were further tested using the same procedure outlined above using higher concentrations of mefenoxam (200 µg/ml) and fosetyl-Al (500 and 3,745 µg/ml); the former fosetyl-Al rate was based on the label recommendation for soil drenches used to combat Phytophthora root rot in nonbearing avocado. Additional fungicides dimethomorph (200 µg/ml), dimethomorph + ametoctradin (400 µg/ml), and fluoxastrobin (100 µg/ml) were also used.

Analyses

Each experiment was conducted twice, and data were compared among the trials using analysis of variance (ANOVA). Data were combined when ANOVA did not find significant differences; otherwise, the entire experiment was repeated. The Tukey–Kramer honestly significant difference test was used to separate the means of RG among different variables such as isolate genotype, isolate origin (nursery versus forest), isolate source (soil versus infected tissue), host genera, and host symptom (symptomatic versus

TABLE 1
Active ingredients of five fungicides used to assess sensitivity of *Phytophthora* present in Maryland's ornamental nursery production and mid-Atlantic oak forests

Active ingredient	Trade name	FRAC code[a]	Label rate	Risk[b]
Mefenoxam	Subdue Maxx (Syngenta)	4	19–37 µg/ml	High
Fosetyl-Al	Aliette WDG (Bayer)	P07[c]	3,745 µg/ml[d]	Low
Dimethomorph	Stature SC (BASF)	40	239–479 µg/ml	Low to medium
Dimethomorph + ametoctradin	Orvego (BASF)	40 + 45	0.5–1.1 µg/ml	Medium to high[e]
Fluoxastrobin	Disarm 480 SC (Arysta LifeScience)	11	5.6–22.5 µg/ml	High

[a] Fungicide Resistance Action Committee (FRAC) (https://www.frac.info/home).
[b] Risk of chemical resistance according to the FRAC code.
[c] Reclassified from U33 in 2018.
[d] Label rate based on soil drench used to combat Phytophthora root rot in nonbearing avocado.
[e] Risk listed is for ametoctradin (FRAC code 45).

asymptomatic). Significant differences were set at $P \leq 0.05$, and the software JMP 10.0.2 (SAS Institute, Cary, NC) was used for all statistical evaluations.

Fungicide Sensitivity

Of the five species tested (in total 243 isolates), most isolates were intermediately sensitive to mefenoxam at a concentration of 100 µg/ml (Table 2). *P. plurivora* had the greatest number of insensitive isolates (12%), followed by *P. multivora* (11%) and *P. cinnamomi* (4%); *P. citrophthora* and *P. pini* had no insensitive isolates (Table 2). All *P. pini* isolates tested were intermediately sensitive to the fungicide.

Isolates were also tested on the off-label fosetyl-Al concentration (100 µg/ml). Unsurprisingly, none of the isolates were sensitive and a majority were completely insensitive at this concentration (Table 2). In total 28 isolates (15 *P. cinnamomi*, two *P. citrophthora*, one *P. multivora*, one *P. pini*, and nine *P. plurivora*) grew faster (on average 14%) on medium amended with fosetyl-Al than on nonamended controls.

No significant differences were found in RG rates among the mefenoxam-tested isolates when grouped based on isolate origin (forest or nursery) or isolate source (soil, water, or necrotic root or stem tissue) (Supplementary Table 1). Similarly, host was not a significant factor for any of the species except for *P. plurivora*: isolates recovered from *Sophora* spp. had significantly greater rates than those recovered from *Ilex*, *Pieris*, *Quercus*, and *Rhododendron* but not *Acer*, *Buxus*, or *Tilia* spp. For almost all *Phytophthora* species, isolates recovered from asymptomatic hosts (e.g., isolates that were recovered from potting media that included fine roots of randomly selected asymptomatic hosts) had a significantly greater RG rate when compared with isolates recovered from symptomatic hosts (e.g., isolates recovered from lesions or wilted plants). The exceptions were seen only in *P. multivora* and *P. pini* (Supplementary Table 1).

A total of 18 isolates were identified as insensitive to mefenoxam, even at a concentration above the label rate (100 µg/ml) (Table 2). When these isolates were screened on higher concentrations of mefenoxam (200 µg/ml) and fosetyl-Al (500 µg/ml) they remained insensitive (Table 3). However, these concentrations of fosetyl-Al are significantly lower than the label rate, and when these isolates were tested at the label rate (3,745 µg/ml) they were sensitive (Table 3). Dimethomorph and dimethomorph + ametoctradin were similarly effective; most mefenoxam-insensitive isolates were inhibited by these chemicals, and only two *P. plurivora* isolates were insensitive to dimethomorph and dimethomorph + ametoctradin.

Isolates were also grouped based on genetic clusters created using AFLP markers (Beaulieu et al. 2017). The three insensitive *P. cinnamomi* isolates (two from mid-Atlantic forest soil and one from soil surrounding asymptomatic *Pieris japonica* 'Scarlett O'Hara') were in the two smallest clusters (there were four clusters in total; genotypic characterization was based on 102 isolates) (Table 4). Interestingly, the cluster to which two of the insensitive *P. cinnamomi* isolates belonged had a significantly greater average RG rate compared with the other clusters. In contrast, the insensitive isolates of *P. plurivora* were more evenly distributed among four of the six clusters (genotypic characterization was based on 186 isolates). Similar to *P. cinnamomi*, differences in average RG existed among the molecular clusters for *P. plurivora* (Table 4). In particular, isolates that were placed in one of the smaller clusters had significantly greater RG rates compared with isolates grouped within the five other clusters.

Fungicide Sensitivity of *Phytophthora* Isolates from Maryland Nurseries

This study is the first to examine the sensitivity of *Phytophthora* in Maryland ornamental nurseries to commonly used fungicides. The findings provide a greater understanding of what fungicides can be used to effectively manage these pathogens. Screening of the five most common species revealed a wide variety of sensitivity both within and between species. A large proportion of isolates were intermediately insensitive, and in at least three species, mefenoxam-insensitive isolates were identified. This range of sensitivity to mefenoxam has been demonstrated throughout the United States in different species. For example, in Tennessee,

TABLE 2
Isolate distribution based on relative growth (RG) rates on clarified V8 juice agar amended with mefenoxam and fosetyl-Al

Phytophthora spp.	Sensitivity[a]	*n*	Mefenoxam (100 µg/ml)	*n*	Fosetyl-Al (100 µg/ml)
P. cinnamomi	Sensitive	77	17	74	0
	Intermediate		57		6
	Insensitive		3		68
P. citrophthora	Sensitive	23	10	17	0
	Intermediate		13		6
	Insensitive		0		11
P. multivora	Sensitive	18	4	7	0
	Intermediate		12		3
	Insensitive		2		4
P. pini	Sensitive	18	0	13	0
	Intermediate		18		7
	Insensitive		0		6
P. plurivora	Sensitive	107	10	91	0
	Intermediate		84		18
	Insensitive		13		73
Overall	Sensitive	243	41	202	0
	Intermediate		184		40
	Insensitive		18		162

[a] Isolates were considered sensitive if RG (mycelial growth inhibition compared with controls) was 0%, intermediately sensitive if RG = >0 to <50%, and insensitive if RG ≥50%.

P. hydropathica isolates were able to grow as much as 20% on medium amended with 100 µg/ml as they were on nonamended medium, and all *P. citrophthora* and *P. citricola* isolates were insensitive (Hulvey et al. 2010). Similarly, in Virginia, 26% of *P. nicotianae* isolates were highly resistant to mefenoxam concentrations of 100 µg/ml (Hu et al. 2008), and all but 12% of *P. cinnamomi* isolates were highly sensitive to the same concentration of mefenoxam (Hu et al. 2010). Variation in sensitivity was also found in California *P. citricola* and *P. cactorum* populations: all 132 *P. cactorum* isolates were sensitive to mefenoxam at 1 ppm, whereas all but one of 86 *P. citricola* isolates were insensitive at the same concentration. A subset of these isolates was insensitive even up to 100 ppm (Bhat and Browne 2007; Bhat et al. 2006). In Florida, 90% of *Phytophthora* isolates (*P. cinnamomi*, *P. katsurae*, *P. palmivora*, *P. nicotianae*, and *P. tropicalis*) from ornamental plants were sensitive to mefenoxam at 100 µg/ml (Patel et al. 2016). The remaining 10% of isolates (one *P. tropicalis* and three *P. nicotianae* isolates) were insensitive. Similarly, a range of mefenoxam sensitivity was detected in agricultural ecosystems in New York *P. capsici* populations (Dunn et al. 2010), further demonstrating the variability in fungicide sensitivity in *Phytophthora*.

Three of the species used in the current study (*P. pini*, *P. plurivora*, and *P. multivora*) were previously classified as *P. citricola.* This is the first study to differentiate their sensitivity to fungicides

TABLE 3
Sensitivity of mefenoxam-insensitive isolates (based on 100 µg/ml) when tested at higher concentrations of various fungicides

Phytophthora spp.	N[a]	Sensitivity[b]	Dimethomorph (400 µg/ml)	Dimethomorph + ametoctradin (100 µg/ml)	Fluoxastrobin (200 µg/ml)	Fosetyl-Al (500 µg/ml)	Fosetyl-Al (3,745 µg/ml)	Mefenoxam (200 µg/ml)
P. cinnamomi	3	Sensitive	3	3			3	
		Intermediate						
		Insensitive			3	3		3
P. multivora	2	Sensitive	2	2			2	
		Intermediate						
		Insensitive			2	2		2
P. plurivora	13	Sensitive	11	11			13	
		Intermediate						
		Insensitive	2	2	13	13		13

[a] Number of mefenoxam-insensitive isolates (100 µg/ml).
[b] Isolates were considered sensitive if relative growth (RG) (mycelial growth inhibition compared with controls) was 0%, intermediately sensitive if RG = >0 to <50%, and insensitive if RG ≥50%.

TABLE 4
Mean relative growth and standard deviation of isolates when grown in growth medium amended with mefenoxam (100 µg/ml), broken down by genetic clusters[a]

Phytophthora spp.	Genetic cluster[b]	*n*	No. of insensitive isolates	Relative growth (%)	Tukey' HSD[c]
P. cinnamomi	1	16	1	7.2 ± 16	B
	2	10	2	23.5 ± 30	A
	3	21	0	4.5 ± 4	B
	4	30	0	6.9 ± 7	B
P. citrophthora	1	11	0	10.2 ± 6	A
	2	9	0	0.5 ± 1	B
	3	3	0	4.2 ± 7	AB
P. multivora	1	9	1	14.2 ± 21	A
	2	5	0	9.6 ± 12	A
	3	2	0	14.4 ± 17	A
	4	2	1	49.9 ± 68	A
P. pini	1	2	0	10.1 ± 2	A
	2	5	0	21.6 ± 7	A
	3	10	0	19.8 ± 14	A
	4	1	0	3.9	A
P. plurivora	1	19	3	30.7 ± 23	B
	2	9	5	55.4 ± 26	A
	3	11	0	3.4 ± 2	C
	4	25	4	20 ± 26	BC
	5	17	1	20 ± 13	BC
	6	26	0	9.9 ± 6	C

[a] Isolate collection consisted of those recovered from ornamental nurseries and mid-Atlantic oak forests.
[b] Genetic clusters were previously identified based on AFLP markers (Beaulieu et al. 2017).
[c] Means followed by the same letter in a column are not significantly different based on Tukey's honestly significant difference ($P < 0.05$).

and suggest that there is considerable variation among these species. Previous studies testing *P. citricola* isolates in California horticultural operations (Bhat and Browne 2007) and Tennessee nurseries (Donahoo and Lamour 2008; Hulvey et al. 2010) described genotypically diverse populations and isolates insensitive to fungicides. In California, all but one of the 86 isolates were insensitive to mefenoxam at 1 ppm, and only a subset of the isolates was sensitive at 100 ppm. In Tennessee, 10% of a collection of 20 *P. citricola* isolates from symptomatic leaves on woody ornamentals were insensitive to mefenoxam at a concentration of 100 ppm. Several isolates collected from symptomatic leaves in nurseries and watersheds in Tennessee were insensitive to the same concentration of mefenoxam, whereas others were not. Our study further demonstrates the variations that exist within and between species to mefenoxam.

Although several studies report half maximal effective concentration (EC50) values of well below the label rate (Chan and Kwee 1986; Farih et al. 1981; Guest 1984; Matheron and Porchas 2000), isolates in the current study were insensitive to the below-label concentrations of fosetyl-Al. However, all isolates were sensitive to fosetyl-Al at the label rate, demonstrating that on-label concentrations of fosetyl-Al are still useful for managing oomycetes. The fact that our isolates, collected in 2010, were not inhibited by lower, off-label concentrations compared with those described in the literature could suggest a shift in sensitivity over time or a geospatial difference in sensitivity. Indeed, wide interspecific and intraspecific variation in tolerance to phosphite was observed in multiple *Phytophthora* species collected in California and other U.S. states (Hunter et al. 2018).

The concentration of fluoxastrobin used in this study (200 µg/ml) is approximately 10 to 35× higher than the label rate, yet all isolates were insensitive. Unlike mefenoxam and fosetyl-Al, which were both introduced in 1977, fluoxastrobin is relatively new and was introduced in 2000 (Morton and Staub 2008). However, those isolates insensitive to mefenoxam were also insensitive to fluoxastrobin (Table 3). Few studies have tested the efficacy of this chemical on *Phytophthora* spp. in nursery populations.

Dimethomorph alone or combined with ametoctradin was more effective. All but two of the 18 isolates screened were completely sensitive. Dimethomorph was introduced in 1988 (Morton and Staub 2008) but has been used less than mefenoxam and fosetyl-Al by an order of magnitude (Boline et al. 2013; McDaniel et al. 2006). Because ametoctradin is a newer product, future work should include the development of a dose-response curve. This is the first report of insensitivity to dimethomorph in Maryland ornamental nursery *Phytophthora* populations. This finding underscores the need of fungicide resistance management and integrated pest management strategies to enable long-term use of newly developed fungicides.

Resistance to phenylamide fungicides is thought to have originated from naturally occurring insensitive isolates existing at a low proportion in the population before being exposed to these fungicides (Gisi and Cohen 1996). This view is further supported by earlier studies in which tolerance to *P. citricola* and *P. cinnamomi* was recorded at concentrations as low as 0.25 µg/ml from isolates collected over many years all over the world as early as 1927 (Coffey et al. 1984).

We previously hypothesized that forest isolates, having little to no exposure to fungicides, would be more sensitive to fungicides than isolates collected from nurseries. This study shows that natural populations in mid-Atlantic forests already include insensitive isolates (two *P. cinnamomi* isolates) and suggests that repeated exposure to fungicides over time is not the only driver of fungicide resistance evolution.

One interesting finding was the significantly greater RG rates of solates obtained from asymptomatic hosts (isolates obtained from potting media of "healthy looking" plants) compared with symptomatic hosts (isolates were recovered mainly from lesions). This could suggest that differences exist between colonization rates in soil versus in planta and that selection of better colonizers is occurring in the soil. Although we did not test whether nursery isolates with exposure to fungicides are better colonizers of fungicide-treated plant tissue, differences in RG rate of isolates recovered from different hosts suggest the need for in vivo experiments. In Australia, *P. cinnamomi* isolates collected from sites previously treated with phosphite (the active ingredient in fosetyl-Al) were more extensive colonizers of phosphite-threated plant tissue (Dobrowolski et al. 2008).

In this isolate collection, *P. plurivora*, a homothallic species, has a relatively greater genetic diversity compared with *P. cinnamomi*, a heterothallic species (Beaulieu et al. 2017). This could be an explanation for why a greater proportion of insensitive *P. plurivora* isolates was detected. Interestingly, the insensitive isolates were genetically diverse and grouped into four out of six genetic clusters. Our data also indicate that insensitivity to fungicides is linked to genetic makeup, as shown by the significantly greater RG values. Exploring fungicide resistance (in particular metalaxyl and mefenoxam) in connection with isolate genotypes has been the focus of numerous studies (Bhat and Browne 2007; Bhat et al. 2006; Duan et al. 2008; Dunn et al. 2010; Hantula et al. 2000; Keinath 2007; Shattock 1988; Timmer et al. 1998). These studies have shown that metalaxyl resistance is controlled by a single nuclear locus that exhibits incomplete dominance, and as a result, resistance can develop. In vitro selection of resistance to metalaxyl was also demonstrated, which might signify that field resistance to fungicides might evolve as a result of their use (Coffey et al. 1984; Ferrin and Kabashima 1991; Joseph and Coffey 1984).

Mefenoxam-insensitive isolates accounted for 4 to 13% of the Maryland ornamental nursery *Phytophthora* population, depending on the species. Although this number may appear small, these populations can increase rapidly due to the biology of the organism. For example, it was found not only that mefenoxam-resistant *P. nicotianae* isolates were able to outcompete sensitive isolates within three to six sporulation cycles but also that they exhibited a greater infection rate and higher sporulation ability (Hu et al. 2008). Another study showed that, even after 2 years without the application of mefenoxam, isolates remained insensitive to the chemical, indicating that insensitivity does not have significant fitness costs in the absence of the chemical (Lamour and Hausbeck 2001; Timmer et al. 1998). Thus, it should not be taken lightly that only a fraction of isolates was insensitive to the fungicides tested in the current study.

This is the first attempt to characterize the fungicide sensitivity of *Phytophthora* species in Maryland ornamental nurseries. Mefenoxam proved to be ineffective against 7% of isolates tested, and its continued use could lead to the selection of insensitive isolates in nursery populations of *Phytophthora*. The same can be said for fluoxastrobin. The development of dose-response curves for these chemicals will provide more insight into what concentrations may be more effective. A *Phytophthora* fungicide program utilizing these chemicals in a nursery must consider the species of concern, because differences in fungicide tolerance exist among species.

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Literature Cited

Balci, Y., Balci, S., Eggers, J., MacDonald, W. L., Juzwik, J., Long, R. P., and Gottschalk, K. W. 2007. *Phytophthora* spp. associated with forest soils in eastern and north-central U.S. oak ecosystems. Plant Dis. 91:705-710.

Balci, Y., Balci, S., MacDonald, W. L., and Gottschalk, K. W. 2008. Relative susceptibility of oaks to seven species of *Phytophthora* isolated from oak forest soils. For. Pathol. 38:394-409.

Beaulieu, J., Ford, B., and Balci, Y. 2017. Genotypic diversity of *Phytophthora cinnamomi* and *P. plurivora* in Maryland's nurseries and Mid-Atlantic forests. Phytopathology 107:769-776.

Bhat, R. G., and Browne, G. T. 2007. Genetic diversity in populations of *Phytophthora citricola* associated with horticultural crops in California. Plant Dis. 91:1556-1563.

Bhat, R. G., Colowit, P. M., Tai, T. H., Aradhya, M. K., and Browne, G. T. 2006. Genetic and pathogenic variation in *Phytophthora cactorum* affecting fruit and nut crops in California. Plant Dis. 90:161-169.

Bienapfl, J. C., and Balci, Y. 2014. Movement of *Phytophthora* spp. in Maryland's nursery trade. Plant Dis. 98:134-144.

Boline, D., Dawson, A., Harris, H., Huff, L., Nice, B., and Suljevic, A. 2013. Maryland pesticide statistics for 2011. https://mda.maryland.gov/Documents/MD_Pesticide_Stats_2011.pdf.

Chan, L. U. Z. G., and Kwee, L. I. M. T. 1986. Comparative in vitro sensitivity of selected chemicals on *Phytophthora palmivora* from cocoa and durian. Pertanika 9:183-191.

Coffey, M. D., Klure, L. J., and Bower, L. A. 1984. Variability in sensitivity to metalaxyl of isolates of *Phytophthora cinnamomi* and *Phytophthora citricola*. Phytopathology 74:417-422.

Cohen, Y., and Coffey, M. D. 1986. Systemic fungicides and the control of oomycetes. Annu. Rev. Phytopathol. 24:311-338.

Daughtrey, M. L., and Benson, D. M. 2005. Principles of plant health management for ornamental plants. Annu. Rev. Phytopathol. 43:141-169.

Dobrowolski, M. P., Shearer, B. L., Colquhoun, I. J., O'Brien, P. A., and Hardy, G. E. S. J. 2008. Selection for decreased sensitivity to phosphite in *Phytophthora cinnamomi* with prolonged use of fungicide. Plant Pathol. 57:928-936.

Donahoo, R. S., and Lamour, K. H. 2008. Characterization of *Phytophthora* species from leaves of nursery woody ornamentals in Tennessee. HortScience 43:1833-1837.

Duan, C. H., Riley, M. B., and Jeffers, S. N. 2008. Characterization of *Phytophthora cinnamomi* populations from ornamental plants in South Carolina, USA. Arch. Phytopathol. Plant Prot. 41:14-30.

Dunn, A. R., Milgroom, M. G., Meitz, J. C., McLeod, A., Fry, W. E., McGrath, M. T., Dillard, H. R., and Smart, C. D. 2010. Population structure and resistance to mefenoxam of *Phytophthora capsici* in New York State. Plant Dis. 94:1461-1468.

Farih, A., Tsao, P. H., and Menge, J. A. 1981. Fungitoxic activity of efosite aluminum on growth, sporulation, and germination of *Phytophthora parasitica* and *P. citrophthora*. Phytopathology 71:934-936.

Ferrin, D. M., and Kabashima, J. N. 1991. In vitro insensitivity to metalaxyl of isolates of *Phytophthora citricola* and *P. parasitica* from ornamental hosts in Southern California. Plant Dis. 75:1041-1044.

Gisi, U., and Cohen, Y. 1996. Resistance to phenylamide fungicides: A case study with *Phytophthora infestans* involving mating type and race structure. Annu. Rev. Phytopathol. 34:549-572.

Guest, D. I. 1984. The influence of cultural factors on the direct antifungal activities of fosetyl-al, propamocarb, metalaxyl, SN 75196 and Dowco 444. J. Phytopathol. 111:155-164.

Hantula, J., Lilja, A., Nuorteva, H., Parikka, P., and Werres, S. 2000. Pathogenicity, morphology and genetic variation of *Phytophthora cactorum* from strawberry, apple, rhododendron, and silver birch. Mycol. Res. 104:1062-1068.

Hu, J. H., Hong, C. X., Stromberg, E. L., and Moorman, G. W. 2008. Mefenoxam sensitivity and fitness analysis of *Phytophthora nicotianae* isolates from nurseries in Virginia, USA. Plant Pathol. 57:728-736.

Hu, J. H., Hong, C. X., Stromberg, E. L., and Moorman, G. W. 2010. Mefenoxam sensitivity in *Phytophthora cinnamomi* isolates. Plant Dis. 94: 39-44.

Hulvey, J., Gobena, D., Finley, L., and Lamour, K. 2010. Co-occurrence and genotypic distribution of *Phytophthora* species recovered from watersheds and plant nurseries of eastern Tennessee. Mycologia 102:1127-1133.

Hunter, S., Williams, N., McDougal, R., Scott, P., and Garbelotto, M. 2018. Evidence for rapid adaptive evolution of tolerance to chemical treatments in *Phytophthora* species and its practical implications. PLoS One 13: e0208961.

Hwang, J., and Benson, D. M. 2005. Identification, mefenoxam sensitivity, and compatibility type of *Phytophthora* spp. attacking floriculture crops in North Carolina. Plant Dis. 89:185-190.

Joseph, M. C., and Coffey, M. D. 1984. Development of laboratory resistance to metalaxyl in *Phytophthora citricola*. Phytopathology 74:1411-1414.

Keinath, A. P. 2007. Sensitivity of populations of *Phytophthora capsici* from South Carolina to mefenoxam, dimethomorph, zoxamide, and cymoxanil. Plant Dis. 91:743-748.

Lamour, K. H., and Hausbeck, M. K. 2001. The dynamics of mefenoxam insensitivity in a recombining population of *Phytophthora capsici* characterized with amplified fragment length polymorphism markers. Phytopathology 91:553-557.

Landschoot, P., and Cook, J. 2005. Sorting out the phosphonate products. Golf Course Manage. 83:73-77.

Matheron, M. E., and Porchas, M. 2000. Impact of azoxystrobin, dimethomorph, fluazinam, fosetyl-Al, and metalaxyl on growth, sporulation, and zoospore cyst germination of three *Phytophthora* spp. Plant Dis. 84: 454-458.

McConnell, M. E., and Balci, Y. 2014. *Phytophthora cinnamomi* as a contributor to white oak decline in mid-Atlantic United States forests. Plant Dis. 98:319-327.

McDaniel, J., Harris, H., Hyatt, R., Taylor, B., and Noguerra, G. 2006. Maryland pesticide statistics for 2004. https://mda.maryland.gov/Documents/MD_Pesticide_Stats_2004.pdf.

Morton, H., and Urech, P. 1987. History of the development of resistance to phenylamides fungicides. Page 133 in: Fungicide Resistance in North America. C. J. Delp, ed. American Phytopathological Society, St. Paul, MN.

Morton, V., and Staub, T. 2008. A short history of fungicides. APSnet Feature. https://www.apsnet.org/edcenter/apsnetfeatures/Pages/Fungicides.aspx.

Olson, H. A., and Benson, D. M. 2011. Characterization of *Phytophthora* spp. on floriculture crops in North Carolina. Plant Dis. 95:1013-1020.

Olson, H. A., and Benson, D. M. 2013. Host specificity and variations in aggressiveness of North Carolina isolates of *Phytophthora cryptogea* and *P. drechsleri* in greenhouse ornamental plants. Plant Dis. 97:74-80.

Parra, G., and Ristaino, J. B. 2001. Resistance to mefenoxam and metalaxyl among field isolates of *Phytophthora capsici* causing Phytophthora blight of bell pepper. Plant Dis. 85:1069-1075.

Patel, J. S., Vitoreli, A., Palmateer, A. J., El-Sayed, A., Norman, D. J., Goss, E. M., Brennan, M. S., and Ali, G. S. 2016. Characterization of *Phytophthora* spp. isolated from ornamental plants in Florida. Plant Dis. 100:500-509.

Pérez-Sierra, A., Álvarez, L. A., Vercauteren, A., Heungens, K., and Abad-Campos, P. 2011. Genetic diversity, sensitivity to phenylamide fungicides and aggressiveness of *Phytophthora ramorum* on *Camellia*, *Rhododendron* and *Viburnum* plants in Spain. Plant Pathol. 60:1069-1076.

Shattock, R. C. 1988. Studies on the inheritance of resistance to metalaxyl in *Phytophthora infestans*. Plant Pathol. 37:4-11.

Timmer, L. W., Graham, J. H., and Zitko, S. E. 1998. Metalaxyl-resistant isolate of *Phytophthora nicotianae*: Occurrence, sensitivity, and competitive parasitic ability on citrus. Plant Dis. 82:254-261.

Twizeyimana, M., McDonald, V., Mayorquin, J. S., Wang, D. H., Na, F., Akgül, D. S., and Eskalen, A. 2013. Effect of fungicide application on the management of avocado branch canker (formerly Dothiorella canker) in California. Plant Dis. 97:897-902.

USDA NASS. 2018. Census Data. https://www.nass.usda.gov/AgCensus/index.php.